AL-TR-1991-0004

BIOPHYSICAL TECHNIQUES FOR EXAMINING METABOLIC, PROLIFERATIVE, AND GENETIC EFFECTS OF MICROWAVE RADIATION

Martin L. Meltz

University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, TX 78284-7800

OCCUPATIONAL AND ENVIRONMENTAL HEALTH DIRECTORATE
Brooks Air Force Base, TX 78235-5000

September 1991

Final Report for Period 1 October 1989 - 30 August 1990

Approved for public release; distribution is unlimited.

AIR FORCE SYSTEMS COMMAND
BROOKS AIR FORCE BASE, TEXAS 78235-5000

NOTICES

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility or any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

JOHNATHAN L. KIEL, Ph.D.
Project Scientist

DAVID N. ERWIN, Ph.D.
Chief, Radiofrequency Radiation Branch

MICHAEL L. BINION, Lt Col, USAF, BSC
Chief, Directed Energy Division

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1991	Final 1 October 1989 - 30 August 1990

4. TITLE AND SUBTITLE

Biophysical Techniques for Examining Metabolic, Proliferative, and Genetic Effects of Microwave Radiation

5. FUNDING NUMBERS

C - F33615-87-D-0626
PE - 61102F
PR - 2312
TA - W1
WU - 23

6. AUTHOR(S)

Martin L. Meltz

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, TX 78284-7800

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Armstrong Laboratory
Occupational and Environmental Health Directorate
Brooks Air Force Base, TX 78235-5000

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

AL-TR-1991-0004

11. SUPPLEMENTARY NOTES

Armstrong Laboratory Technical Monitor: Dr. Johnathan L. Kiel, (512) 536-3583

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

This project was undertaken to prepare for a comprehensive research effort examining metabolic, proliferative, and genetic effects of microwave radiation. To accomplish this task, preliminary studies have been performed with 4 cell systems; Chinese hamster ovary (CHO) cells, AS52 Chinese hamster cells [heterozygous at the xanthine-guanine phosphoribosyl transferase (XGPRT) locus], 244B proliferating human lymphoblastoid cells, and freshly isolated peripheral lymphocytes. The thermal response of the 244B cells has been carefully examined, and an initial characterization of the membrane markers, membrane permeability, and cell cycle distribution of these cells undertaken. The absence of the induction of chromosome aberrations in CHO cells, after exposure to 850 MHz pulsed wave (PW), 18 mW/cm^2 (specific absorption rate (SAR) 14.4 W/kg) radiofrequency radiation (RFR), or after exposure to 1,200 MHz PW (220 W - 300 W net forward power; SAR 24.33 W/kg) RFR, is reported. The survival response of the AS52 cells, after simultaneous treatment at 37 °C or 40 °C, with and without mitomycin or Adriamycin, is described. The survival of the AS52 cells after X-ray exposure at low and high dose rates is also described.

14. SUBJECT TERMS		15. NUMBER OF PAGES
Microwave radiation	Radiation biology	40
Lymphocytes	Cell biology	16. PRICE CODE
	Chromosomes	

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

TABLE OF CONTENTS

List of Figures

List of Tables

BIOPHYSICAL TECHNIQUES FOR EXAMINING METABOLIC, PROLIFERATIVE, AND GENETIC EFFECTS OF MICROWAVE RADIATION

INTRODUCTION

Upon examining the effects of microwave radiation on mammalian cells, one can select one biological/biochemical endpoint, and use that as the experimental focus. This focus would be most appropriate when evidence is present to suggest an impact of microwave radiation on that endpoint, with this requiring verification. Alternatively, if the effect had already been conclusively demonstrated, an investigation of its mechanism would be called for.

In our previous research, because of the suggestion in the early literature that microwave radiation can cause genetic damage, we focused on genetic effects. These investigations included studies of the induction of deoxyribonucleic acid (DNA) repair (Meltz et al. 1987), sister chromatid exchange (SCE) (Ciaravino et al., 1987), chromosome aberrations (Kerbacher et al., 1990), and mammalian cell mutation (Meltz et al., 1989). In performing those experiments, considerable attention was focused on control and monitoring of the experimental exposure system, and on microwave and thermal dosimetry (Meltz et al., 1988).

The mammalian cell is a complicated organism with many structural, functional, and metabolic activities existing and occurring; any of these activities can potentially be altered by the deposition of the energy of microwave radiation. While any effects which might be observed are most likely to be the result of a thermal mechanism, nonthermal effects must always be considered a possibility. In addition, thermal effects occurring might not be due (alone) to the final temperature achieved and the time the system is at that temperature, but also to the rate at which that temperature is initially achieved.

In preparation for a comprehensive research effort examining metabolic, proliferative, and genetic effects of microwave radiation, we have performed a series of preliminary investigations. These investigations used 4 cell systems studied in vitro: a Chinese hamster ovary (CHO) cell line; a CHO cell line, designated AS52, which is heterozygous at the Xanthine-guanine phosphoribosyl transferase (XGPRT) locus; the 244B human lymphoblastoid cell line (of B cell origin); and freshly isolated human peripheral lymphocytes.

Using these cell systems, we have initiated studies of their thermal response, chemical response, and ionizing radiation response (at high and low dose rates); of characterization of membrane markers; and of adaption of an air incubator system to our experimental requirements. These preliminary studies will allow a comprehensive investigation of the effects of simultaneous exposures to microwave radiation and ionizing radiation, or selected chemicals.

MATERIALS AND METHODS

Cell Cultures

CHO (10B5) Cells

These cells were reconstituted periodically from frozen cell stock. An ampoule (stored in liquid nitrogen) was rapidly thawed and added directly to 8 ml of warm complete McCoy's 5A medium supplemented with 10% fetal bovine serum (heat inactivated) and 40 μg/ml gentamycin. After a 4-h incubation at 37 °C in a humidified 95% air/5% CO_2 incubator, the attachment medium (containing the initial freezing medium) was aspirated, and 5 ml of fresh complete McCoy's medium was added. The cells were passaged 3 times per week, using a standard trypsinization procedure for cell detachment. They were seeded at passage at 500,000 cells per T-25 flask, or 1×10^6 cells per T-75 flask. The population doubling time is approximately 14 h.

Chinese Hamster Ovary Cells (For Chromosome Aberration Studies)

This cell line was used in the chromosome aberration induction experiments. The cells were maintained as monolayer cultures in T-25 flasks in Ham's F-10 medium supplemented with 10% fetal calf serum and gentamycin.

AS52 Cell Line

The AS52 line of CHO cells was maintained as just described, except that the medium used was Ham's F12 containing 10% heat inactivated fetal calf serum and 40 μg/ml gentamycin.

244B Human Lymphoblastoid Cells

A transformed normal human B-lymphocyte cell line (244B) was kindly donated by Dr. J.L. Schwartz (University of Chicago). This lymphoblastoid cell line was maintained in exponential growth in RPMI-1640 medium supplemented with 20% fetal bovine serum, 1 mM α-ketoglutarate, 3 mM L-glutamine, 0.5 mg/ml gentamycin and 60 units/ml nystatin (complete medium). Cells were seeded at 5×10^5 cells/ml into 75 cm^2 tissue culture flasks and passaged every 48 h by counting and dilution. Cells were also stored frozen in liquid nitrogen for periodic reconstitution.

Human Peripheral Lymphocytes

Human peripheral lymphocytes were isolated from whole blood drawn from volunteer subjects using Ficoll-Paque gradient sedimentation. Briefly, heparinized blood was layered on a Ficoll-Paque solution (Pharmacia Inc, N.J.) and centrifuged at 400X g for 40 min. The lymphocytes separated from the other blood elements and were found at the interface between the plasma and the Ficoll-Paque. They were recovered from the interface using a Pasteur pipette and transferred to a second centrifuge tube before washing 3X with a balanced salt solution. The cell density was determined by hemocytometer count and 2.5×10^6 cells were seeded into 25 cm^2 tissue culture flasks in 5 ml RPMI medium supplemented with 10% fetal bovine serum.

Cell Survival After X-ray, Chemical, and Hyperthermia Treatments

Radiation Exposure Protocol

The cells were detached from the stock culture flask surface using a standard trypsinization technique, counted (by Hemocytometer or Coulter Counter), and plated into T-25 flasks 24 h before radiation exposure. For each dose level, 8 replicate flasks were seeded with a predetermined number of cells (to allow an appropriate number of surviving colonies at each dose level). Additional flasks were seeded with 5,000 cells for multiplicity determination. Immediately before exposure, the attachment medium was removed, and the flasks were filled completely with fresh warm complete medium. They were then irradiated, 4 at a time, in a vertical downward direction using a Maxitron Orthovoltage X-ray unit. The surface with the attached cells faced the X-ray source. The unit was operated at 250 kVp, 20 mA, at a distance which resulted in a dose rate of 1 Gy/min. The filtration was 0.25 mm Al. Additionally, experiments were performed at the dose rate of 0.25 Gy/min, with the X-ray unit operating at 250 kVp, 3 mA, and filtration of 0.25 mm Al. Following exposure, the medium in the flasks was decanted and replaced with 5 ml of fresh prewarmed medium. The flasks were then returned to the 37 ^{o}C incubator, where they remained undisturbed for 7 days to allow for colony formation. Colonies were fixed and stained with crystal violet prepared in 95% ethanol. To determine cellular multiplicity, 2 additional flasks of cells were fixed with 3:1 methanol: glacial acetic acid at the beginning and end of treatment; the multiplicity was calculated as the average number of cells per colony forming unit. The surviving fraction was corrected for plating efficiency (80-90%) and cellular multiplicity at the time of treatment.

Chemical Treatment of AS52 Cells

For chemical experiments, cells were detached from stock flasks and distributed at preselected cell densities into 8 replicate flasks (or a total of 10 replicate flasks at 2 different cell densities) 4 h before chemical treatment. After incubation for cell attachment, a 0.1-ml aliquot of a concentrated chemical stock solution (sterile filtered and serially diluted) was added to each flask; the flasks in each set were gently swirled after addition. The final volume was 5 ml. The cells were returned to the 37 ^{o}C humidified 95% air/5% CO_2 incubator, with caps loosened, and incubated (stationary) for 2 or 4 h. At the designated time, each set of flasks was removed from the incubator, the treatment medium was aspirated, the attached cells were quickly rinsed one time with Puck's Saline G, and then washed more slowly with Puck's Saline G as the other sets (concentrations) of treated cells at that time were then quick-rinsed. After all of the sets at that treatment time were rinsed free of chemical, the saline was aspirated, and fresh warm complete medium was added to each of the flasks. They were then incubated at 37 ^{o}C for 8 days. The cell colonies were then fixed and stained. The colony count was corrected for multiplicity and control cloning efficiency to determine cell survival, as described earlier. The chemicals used in these studies were Adriamycin and mitomycin C. Subsequent to the earlier initial range-finding experiments, a similar chemical treatment protocol was followed, using each chemical, but for 2 preselected concentrations (only) at each period (2 or 4 h). These experiments were designed to give survival levels of chemical treatment alone, which would allow for acceptable survival when simultaneous microwave and chemical treatments were performed.

Hyperthermia Treatment of Surface Attached Cells

In performing hyperthermia treatments of surface attached cells, special precautions were taken to avoid any trypsin heating interaction effect. To achieve this result, an appropriate amount of CHO cells growing surface attached in flasks were detached using trypsin, and inoculated into domed spinner flasks for overnight incubation in suspension. The cell number increased during the 24-h incubation period. At that time, the cells were additionally disaggregated to obtain a single cell suspension using a syringe and 22-g needle; they were then counted. Based on the expected cloning efficiency after treatment, serial dilutions were then prepared for plating at preselected cell densities. The cells were allowed to attach to flask surfaces in T-25 flasks seeded 24 h earlier with a feeder layer of lethally irradiated cells. The attachment period was for 90 min at 37 °C. After 90 min, the caps and necks were sealed by immersion in melted wax, racked, and immersed (completely) in a 37 °C water bath. Beginning at 120 min (after plating), the racks of flasks were immersed in the water baths, preset at the selected temperatures, for the selected heating times. All flasks had to be at 37 °C for at least 5 min before heat treatment (even those most recently waxed). Two flasks were fixed for multiplicity determination at the beginning and end of the series of heat treatments. As the racks of flasks were removed from the heating baths, they were immediately immersed for at least 5 min in the 37 °C water bath. They were then removed from their rack, dried, and placed in the 37 °C incubator for colony growth and cloning efficiency determination.

Flow Cytometry Studies with 244B Cells

Cell Cycle Distribution of 244B Cells

For these experiments, cells which were grown in complete RPMI-1640 medium were spun down and fixed in 70% ice cold ethanol. The DNA content of the cells was then determined using the DNA intercalating dye propidium iodide in a citrate buffer (Krishan, 1975). The amount of stain present is proportional to the amount of DNA present within the cell, which is correlated with specific stages of the cell cycle (e.g., G_0/G_1,S,G_2/M). Flow cytometric analysis of the stained cells yielded histograms demonstrating the number of cells and their relative DNA content. Comparison of the histograms of control and treated cells would yield information on changes of the cell cycle distribution as a result of each treatment protocol.

Analysis of Proportion of Cells in Active DNA Synthesis

Another approach to determining the effects of different exposures on the cell cycle is to examine the effect on cells actively synthesizing DNA. This examination is accomplished by labeling the DNA with bromodeoxyuridine (BrdU). Immediately after exposure, the cells would be incubated with 1 mM BrdU in the growth medium for 18 h. Only cells actually synthesizing DNA incorporate the BrdU into the DNA; this technique will allow a more precise quantification of the effect of exposure (treatment) on proliferating S-phase cells. The staining to determine the incorporation of BrdU requires a more involved protocol than that required for staining total DNA. After the cells are fixed in buffered alcohol, they are washed with phosphate-buffered saline (PBS) and then incubated in 1N HCl for 30 min to allow partial degradation of the DNA (this step allows the later staining of the BrdU directly by monoclonal

antibodies). Following the incubation in acid, the cells are again washed with PBS, and incubated in a buffer solution containing anti-BrdU antibody. The excess antibody is removed and an antibody tagged with a fluorescent compound is added to the cells in a buffered solution. The cells are again washed to remove excess antibody and incubated with 1 ml of propidium iodide in buffered saline (20 μg/ml). The cells are finally analyzed on the flow cytometer, and a comparison of histograms for each exposure (treatment) group is made, giving a more accurate determination of any alteration in DNA synthesis in S-phase cells.

Alternative Fixation Protocol for Flow Cytometry

Cells were originally fixed in 70% ethanol for these studies. However, it soon became clear that this method of fixation was inadequate for our purposes. Loss of signal homogeneity from the fluorescence of the propidium iodide caused a broadening of the peaks in the histograms. It was, therefore, necessary to use a new fixation protocol. After several attempts, a suitable alternative was discovered, i.e., the use of 95% ethanol diluted 1:1 with sodium citrate buffer (50 mM). This effort resulted in an increase of signal homogeneity by a factor of 2 to 3. Figure 1 demonstrates the histogram of 244B cells fixed in ice cold 70% ethanol. Figure 2 demonstrates the same cells fixed in buffered ethanol.

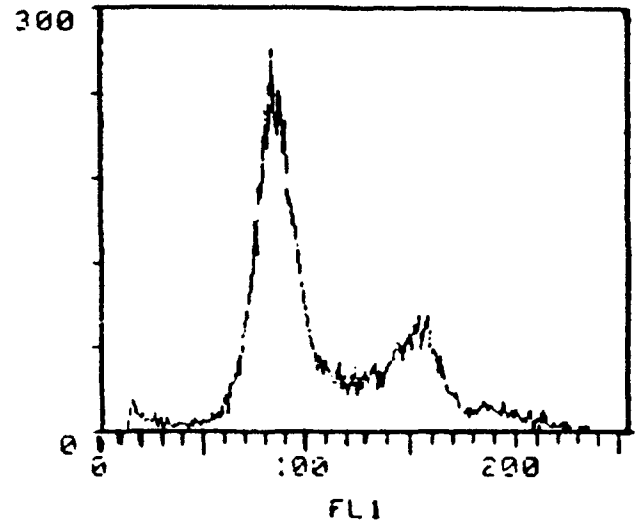


Figure 1. Single parameter histogram of propidium iodide stained DNA of 244B cells fixed in ice cold ethanol. Note peak width.

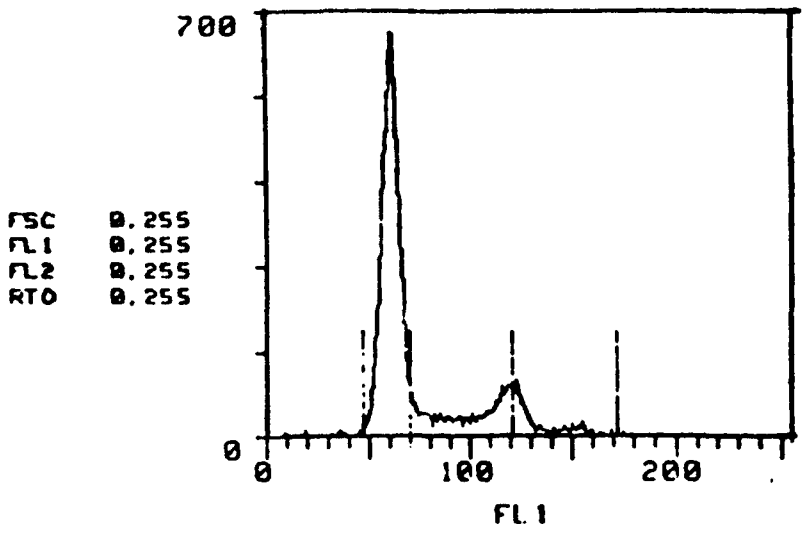


Figure 2. Single parameter histogram of propidium iodide stained DNA of 244B cells fixed in 95% ethanol diluted 1:1 in 50 mM Sodium Citrate Buffer. Note peak width.

Convection Heating of 244B Lymphoblastoid Cells for Studying Effects of Heat on the Cell Cycle

In the preliminary studies, heating was done with controlled temperature water baths for various times and temperatures. The cells were heated in 25 cm^2 tissue culture flasks at a cell density of 2.5 x 10^6 cells per ml with 5 ml medium per flask Early experiments at determining the effects of heat on the cell cycle involved varying heating times at temperatures from 37 ^oC to 43.5 ^oC. From these experiments, we learned that 70% ethanol was inadequate for our fixation needs. In a later experiment, 244B cells were heated at 37, 42, 42.5, 43 and 43.5 ^oC for 1, 2 or 4 h, and then allowed to recover for 0, 18, 24, 48 or 72 h before fixation for flow cytometry analysis.

Surface Marker Analysis on the 244B Lymphoblastoid Cell

The 244B cell line was originally chosen for our study because it was of human origin and purportedly of lymphoblast origin. The use of this type of cell would allow us to determine what effects, if any, our exposure (treatment) had on a relatively nondifferentiated cell; this could act as an indicator of the effects we might expect after exposures of freshly isolated human peripheral lymphocytes of the immune system. The 244B cell line is an Epstein-Barr virus transformed peripheral lymphocyte. The cell was reportedly transformed to the B-lymphoblast stage (Schwartz, J., personal communication). As no pertinent information was available concerning the specific markers present on the surface of these cells when we received them, we tested the cell line using a standard antigen panel designed to identify cells associated with chronic lymphocytic leukemia (B cell, CLL). The tests were run on the Department of Pathology (UTHSCSA) FACScan flow cytometer. The latter is a multilaser system, allowing ease of protocols involving multiple stain analysis. The following is a list of the cluster of differentiation antigens assayed by this panel: CD 2-5 (B CLL), 7, 10, 11C, 13-16, 19-22, 25, and 33.

Additional surface markers assayed included HLA-DR, polyclonal Igs, Immunoglobulins M, D, A, and G, as well as the associated light chain molecules **kappa** and **lambda**. The presence of megakaryctic markers was also tested using PLT-1.

Membrane Permeability

Our preliminary experimental design for studying the effects of simultaneous low dose rate X-ray exposure and microwave exposure calls for exposure times of 4 h. To study the effects of the exposure on membrane permeability, our initial protocol required loading a dye into the cells for the duration of the exposure, followed by flow cytometric analysis to determine whether the exposed cells had lost more dye than the controls (or in fact retained the dye longer than controls). To determine if this design was feasible, it was first necessary to determine the baseline dye loading characteristics of the 244B cell line. Cells in mid-log growth phase were centrifuged; the medium was decanted and the cells were resuspended in 1 ml of PBS. To this solution was added 200 μl of a 300 μg/ml solution of carboxyfluorescein in PBS (pH 8.2). The cells were then incubated for varying periods of time to determine the optimum loading time for the dye, and also to determine the feasibility of long duration exposure experiments. The cells were assayed after 15, 20, 35, 50, and 70 min of incubation with the dye. For controls, both unlabeled cells and dye only (in solution) were run on the flow cytometer. After the dye uptake incubation, the cells were centrifuged and the loading buffer decanted. The cells were than resuspended in 1 ml of PBS and run on a FACS 420 flow cytometer, using a Spectra Physics argon/krypton laser operating at 488 nm and 210 mW. The flow rate was set at 100 cells per second. The side scatter signal was passed through a 530-nm band pass filter and collected on a photo-multiplier tube (PMT) operating at 490 V in the log mode. The data was collected in the list mode, and analyzed on a Consort 30 system.

X-ray Dosimetry

A significant problem associated with our proposed experimental design for simultaneous X-ray and MW exposures (using the G.E. Maxitron Orthovoltage X-ray unit) was the sample position. The samples were to be contained within an air flow incubator positioned on top of 10.2 cm (4-in) thick Echosorb squares (2-ft (61 cm) x 2-ft (61 cm)) on a Plexiglas sheet placed directly above the X-ray head. This shielding is required to prevent the microwaves from reflecting off the X-ray unit during simultaneous microwave exposure. This sample location also was simultaneously to be in the far field for 2.45-GHz microwave radiation. The distance required for the latter was 1.6 m below the face of the microwave antenna horn located near the Echosorb ceiling of the chamber. The anechoic chamber design allows for the lowering of the X-ray source to a position near the cement floor of the chamber (below the surface of the Echosorb floor panels). However, it was not possible to simultaneously increase the distance from the X-ray head to the sample, in order to use distance as a variable for achieving the lower dose rate desired, without simultaneously raising the sample into the near field. The first approach, therefore, was to use the filtration already located in the head of the X-ray unit. When this approach was tried, the X-rays produced were unable to penetrate the Echosorb and Plexiglas shielding between the sample and the X-ray head. To remedy this problem, a series of experiments were implemented to determine the conditions necessary to lower the dose rate to the samples at

a fixed distance of 1.6 m from the microwave horn, and still maintain sufficient penetration energy to pass through the shielding and irradiate the samples in a homogeneous field.

The final design for low dose rate exposures requires lowering the X-ray unit to its lowest position and placing a 0.5-cm thick sheet of Plexiglas over the unit to support the Echosorb foam pads. These pads are about 10-cm thick and are used in double thickness to ensure that there is no interaction between the microwaves and the X-ray unit. Copper filters of 3.361-mm thickness were placed directly over the port of the X-ray unit in this position. The voltage of the X-ray unit was set at 150 kVp, and the current at 3 mA. The final distance from the X-ray unit to the cells was 69 cm (27-in). Dosimetry was performed using lithium fluoride (LiF) thermoluminescent dosimeters, which were read on a Victoreen M2800 Thermoluminescent Dosimetry (TLD) reader. Exposure data were determined from the integration of the area under the glow curve corresponding to the energy stored in the TLD chips from the X-ray exposures. X-ray field homogeneity was determined by sampling from 5 positions, within each of the sample flasks at each flask position, and also measuring and recording the dose received at the center of the incubator, corresponding to the center of the X-ray field (Fig. 3).

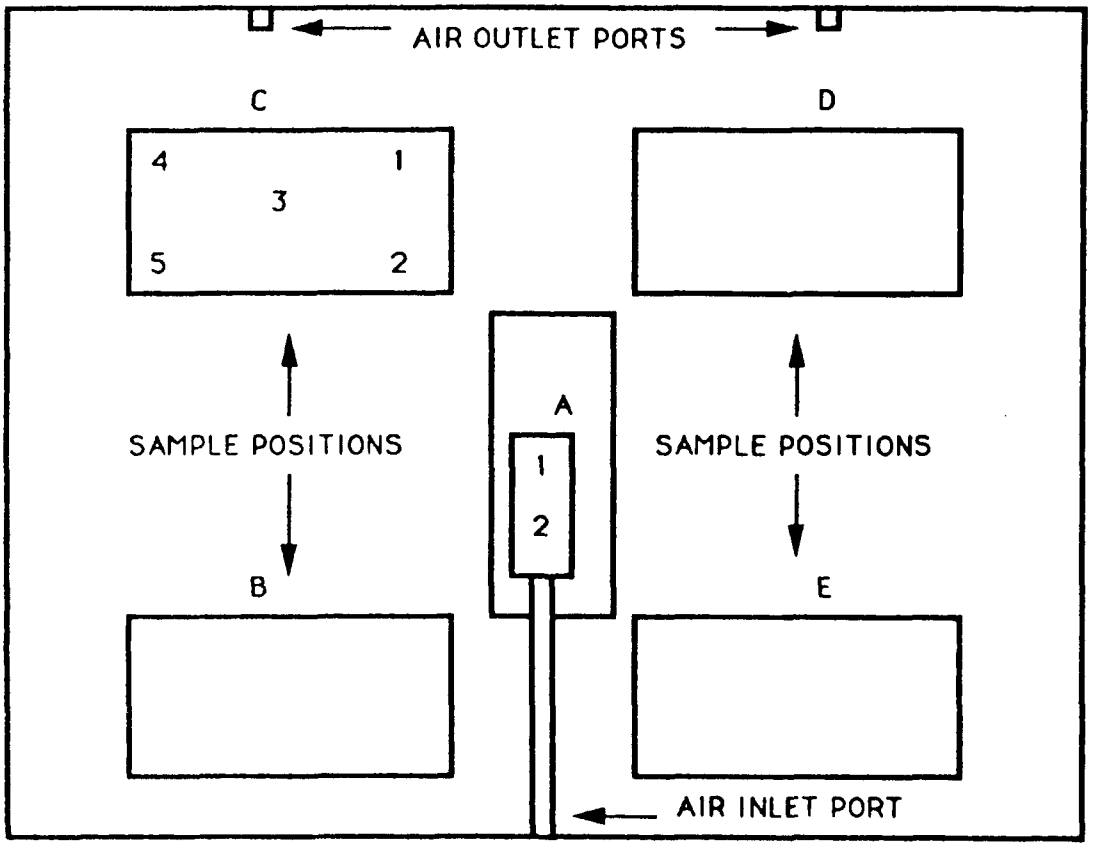


Figure 3. Diagram of the air flow incubator, A through E indicating sample positions. Numbers 1 through 5 indicate arrangement of TLD chips used in each sample position for dosimetry.

Air Flow Incubator

The adaption of this system for use in our laboratory for our experiments became necessary when it was determined that circulating water baths were inadequate to achieve temperatures above 40 °C, in microwave experiments using circulating water and water baths (heated initially to 37 °C). The air flow incubator allows precise control of the temperature of the irradiated samples, without absorbing appreciable amounts of microwave energy (as is the case in the water baths). The basic design is a hollow foam box, containing 4 removable foam inserts for holding tissue culture flasks in an air incubator chamber (Figures 4 and 5). This box is composed of noninteractive foam type material. The air is fed through a central plastic tube, which has ports drilled into it to disperse the air in the chamber.

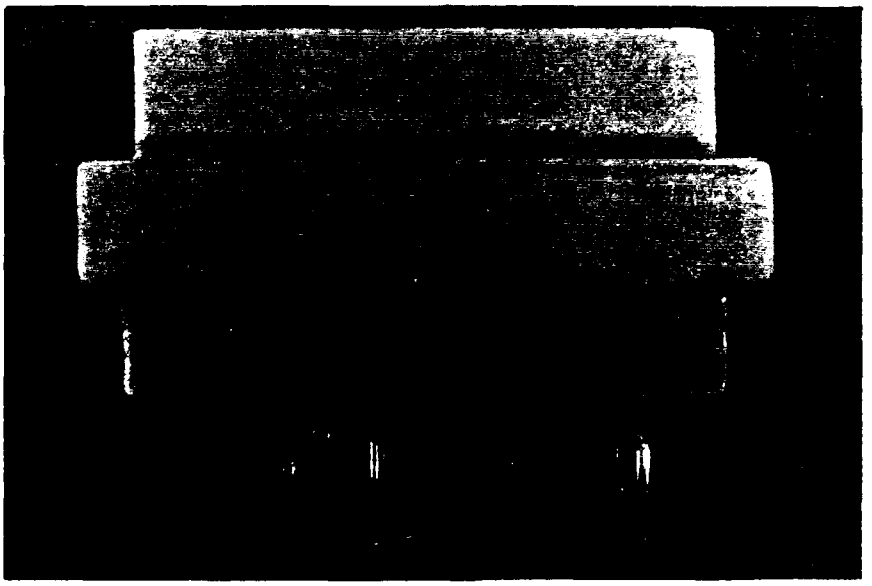


Figure 4. Individual foam insert for the air incubators, showing the attachment of a T-25 flask with Velcro strips. This method allows the suspension of the flask in the air chamber of the incubator. Vitek probe for temperature measurement is shown entering the flask through a hole on its top surface.

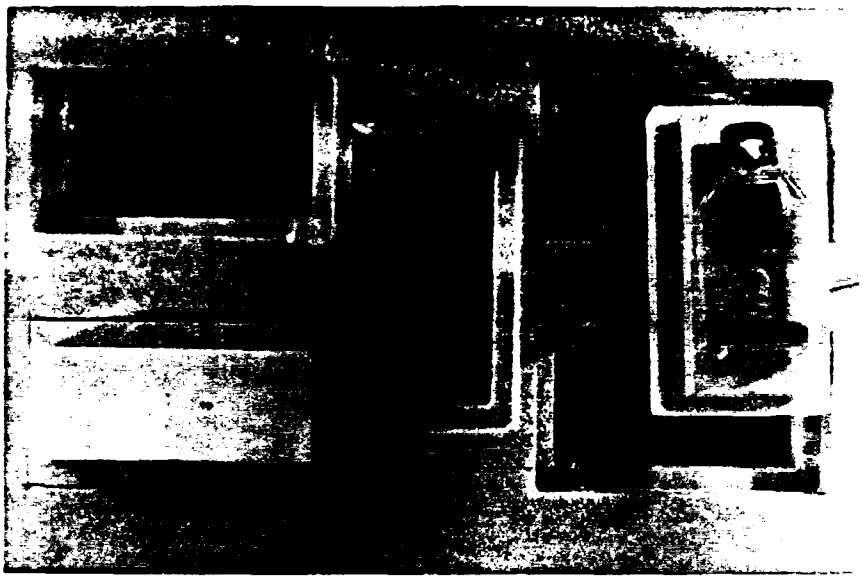


Figure 5. Top view of the air incubator. The 4 horizontal positions would be used to hold flasks with cells during the microwave exposure in the air chamber below; the central position would be used for a reference temperature measurement. The incoming air enters through the tube at the bottom of the air chamber (seen through the center opening).

Chromosome Aberration Studies After RFR Exposure

Radiofrequency radiation (RFR) exposures at 850 and 1,200 MHz were performed in a 12.2 x 6.1 x 3.0 m (40 x 20 x 10 ft) anechoic chamber (No. 2) in the Radiofrequency Research Laboratory at the U.S. Air Force School of Aerospace Medicine, Brooks Air Force Base, TX. The air temperature in the chamber was initially maintained at 37 °C. The pulsed wave (PW) RFR was transmitted from a rectangular antenna horn in a vertically downward direction.

The power densities, specific absorption rates (SARs), mode of exposure, frequency, and duty factor for the PW exposures are described in the following section. The horn to water bath distance was 1.6 m (5.2 ft), thus placing the flasks or dishes containing the cells to be exposed in the near field, just short of the far field. In these chromosome aberration studies, cells were exposed in 25 cm^2 tissue culture flasks (T-25 flasks) immersed in a large square water bath, but positioned as inserts in the bottom side of a continuously rotating circular Styrofoam float. The latter was designed to ensure the homogeneity of the exposure (Meltz et al., 1988).

The temperature was continuously monitored during the RFR exposure period using non-RFR-interactive Vitek probes and a BSD-200 Thermometry system. A rapid rise in medium temperature over the first 20 min in each flask was followed by a slow approach to RFR-induced maximum temperatures at 2 or 4 h approaching 40 °C.

RFR Exposure Parameters

At 850 MHz, for the Chromosome Aberration Studies, the exposure parameters were:

Avg. Net Forward Power:	200 W
Pulse Repetition Rate:	40,000 pps
Pulse Width:	6.5 μs
Duty Factor:	0.26
Power Density:-Avg.	18 mW/cm^2
SAR:	14.4 W/kg

At 1,200 MHz, the exposure parameters were:

Expt. No.:	1	2	3
Avg. Net Forward Power:	300 W	220 W	240 W
Pulse Repet. Rate:	40,000 pps	40,000 pps	40,000 pps
Pulse Width:	6.5 μs	6.5 μs	6.5 μs
Duty Factor:	0.26	0.26	0.26
SAR:			24.33 W/kg

Protocol for the Chromosome Aberration Assay

On the day preceding the exposure, CHO cells were seeded into T-25 flasks and incubated for 24 h at 37 ^{o}C so that they would be nonconfluent and replicating at the time of exposure. Cells were seeded in triplicate for each treatment condition (e.g., RFR exposed, temperature control, 37 ^{o}C control (nonRFR exposed), and positive chemical control). Immediately before the RFR exposure, a small volume of concentrated chemical mutagen (e.g., Adriamycin) was added to selected flasks. All flasks contained 8 ml of complete medium. The flasks were inserted into the circular Styrofoam wheel, which continuously rotated during the RFR exposure (or similarly rotated, and treated as temperature or 37 ^{o}C controls). In these experiments, the RFR exposure was of 2-h duration. Temperature monitoring was continuous during the exposures. At the termination of the RFR exposure, the cells in the flasks were washed with salt solution, and fresh complete Ham's F-10 medium added to each flask. Colcemid (0.02 μg/ml final concentration) was added to each flask, and the cells were incubated for 18 h (allowing mitotic cells to accumulate). At this time, the cell monolayer was washed, the cells were detached, and then fixed in suspension. The cells were then dropped for cytogenetic analysis using standard techniques.

Soft Agar Cloning Procedure for Viability Determination of AS52 Cells

Our ongoing studies of the potential mutagenic interaction between ionizing radiation exposure and microwave radiation use the AS52 cell lines for mutation analysis at the XGPRT locus. In the standard procedure previously described in the literature, viability and selection for mutation always used a surface attachment procedure in tissue culture flasks. In performing this assay, a good deal of labor time was required for passaging the cells every other day in every mutation during the expression period, before addition of the selection chemical (which would result in only mutated

cells being able to form colonies). A recently published protocol (Lee and Rudd, 1990) described an alternative procedure; the treated cells are cloned in soft agar for viability determination, and also in soft agar for expression incubation, before adding the selection chemical. This latter procedure offers a significant improvement over the surface attachment method, in that the every-other-day trypsinizations, counting, and replating of every plate are not required.

In performing the viability part of this procedure, BBL agar was prepared at an initial concentration of 2.2% in medium without serum, and autoclaved to sterilize. The hot agar was then diluted into Ham's F12 medium, resulting in a final 100-ml volume containing 0.22% agar and 2% serum with 600 cells (6 cells/ml). The well-mixed agar with cells was then distributed in 25-ml aliquots into four 20 x 100-mm sterile plastic petri dishes, and allowed to harden for 12 min in a freezer. The petri dishes were then incubated at 37 ^{o}C in a humidified 95% air/5% CO_2 incubator for 7 days, at which time a 15-ml overlay was added to each dish. This overlay consisted of hypoxanthine free Ham's F12 medium with 10% serum and 0.22% agar. The dishes were incubated for another 14-18 days, when they were removed from the incubator. The colonies were stained with thiazolyl blue, and the colonies were counted. This procedure resulted in a very satisfactory control cloning efficiency of 84.2%.

RESULTS

Cell Cycle Analysis Using Flow Cytometry

Figure 6 indicates the relative S-phase of the 244B cells to be about 5.9%. This percentage appears to be low for exponentially growing cells; it is, therefore, likely that the cells examined were late log to early stationary phase cells, which would be expected to have lower values for S.

Effect of Hyperthermia Treatment on 244B Cells Using Flow Cytometry

Data from these experiments (Table 1) indicated, with some variability, that for the 2-h treatment at 42 ^{o}C, there was a decrease of 15% from G_1 at 0 h to G_1 at 18 h. This shift is seen to be reversing by 48 h. After the 42.5 ^{o}C treatment, there is a large shift from G_1 with recovery by 48 h. This shift is most likely due to cell loss and repopulation.

Effect of Hyperthermia on 244B Cell Viability and Growth Kinetics

The results of these investigations have been completed and are summarized in the manuscript entitled: "Hyperthermic Effects on Viability and Growth Kinetics of Human Lymphoblastoid Cells" (Holahan, P.K., Eagan, P., and Meltz, M.L., accepted for publication, Int. J. of Hyperthermia, 1991).

Surface Marker Analysis by Flow Cytometry

Upon screening with the selected panel of surface marker antigens, the 244B cell line tested positive for the following markers: HLA-DR, CD 15, 19, 20, 21; the polyclonal Igs M, D, A, G; and the light chain immuglobulin

TABLE 1. CELL CYCLE DISTRIBUTION OF HEAT-TREATED 244B CELLS

37 °C				42.5 °C (2-h incubation)			
Recovery Time	% G_1	% S	% G_2-M	Recovery Time	% G_1	% S	% G_2-M
0 h	39.4	35	8.8	0 h	41.3	19.4	10.5
18 h	33.7	17.7	19.0	18 h	17.0	27.1	17.8
24 h	44.6	16.9	13.3	24 h	26.3	27.3	10.1
48 h	54.8	16.8	11.8	48 h	44.9	20.7	10.0
72 h	60.1	18.7	13.3				
42 °C (2-h incubation)				43 °C (2-h incubation)			
Recovery Time	% G_1	% S	% G_2-M	Recovery Time	% G_1	% S	% G_2-M
0 h	52.2	15.5	12.5	0 h	50.9	17.2	11.9
18 h	37.1	16.0	18.3	18 h	56.5	13.2	8.9
24 h	35.4	29.5	12.2	24 h	33.5	20.3	21.2
48 h	44.0	25.3	2.6	48 h	31.6	15.9	17.9

associated proteins kappa and lambda (Table 2). HLA-DR is a main histocompatibility antigen and is found on all B cells, monocytes and activated T-cells. The presence of HLA-DR, CD 19, and CD 20 was expected as they are pan-B markers. CD 21 was also expected as it is the surface receptor for the Epstein-Barr virus. Unexpectedly, the granulocytic cell marker CD 15 was present on 59% of the cells tested.

Membrane Permeability Studies

Analysis of the data revealed the following: Excitation of the dye-only solution resulted in only background level fluorescence at the low end of the fluorescence scale (Fig. 7). Exciting only the unlabeled cells resulted in a broad peak around channel 67, which yielded a coefficient of variation (CV) of 24.2 (Fig. 8). The cells incubated with the dye for 15 min yielded 2 distinct peaks, a relatively low broad peak indicative of unlabeled cells located around channel 90, and the main peak of labeled cells located at channel 195 (Fig. 9). This peak contained roughly 80% of the signal and had a peak channel height of 1,251. The data from the 20-min exposure (Fig. 10) yielded a very low peak comprising 3% of the total signal, related to unlabeled cells in the same location as that for the 15-min incubation, but about one-half of the peak height. The main peak, which was located near channel 200, contained 81% of the total signal and had a peak maximum of 1,500. The 35-min (Fig. 11) incubation demonstrated a consistent main peak near channel 160, which had only a small CV (2.3). The peak maximum was about 1,100. The 50-min incubation (Fig. 12) demonstrated 98.5% of the total signal in a single peak near channel 160 (CV 2.3). The 70-min incubation (Fig. 13) showed only a single peak near channel 160; however, the peak was broader than the preceding time points and had a CV of 4.0 and a peak maximum of 643. The summary of the data, for relative peak maxima vs. incubation time, is in Figure 14.

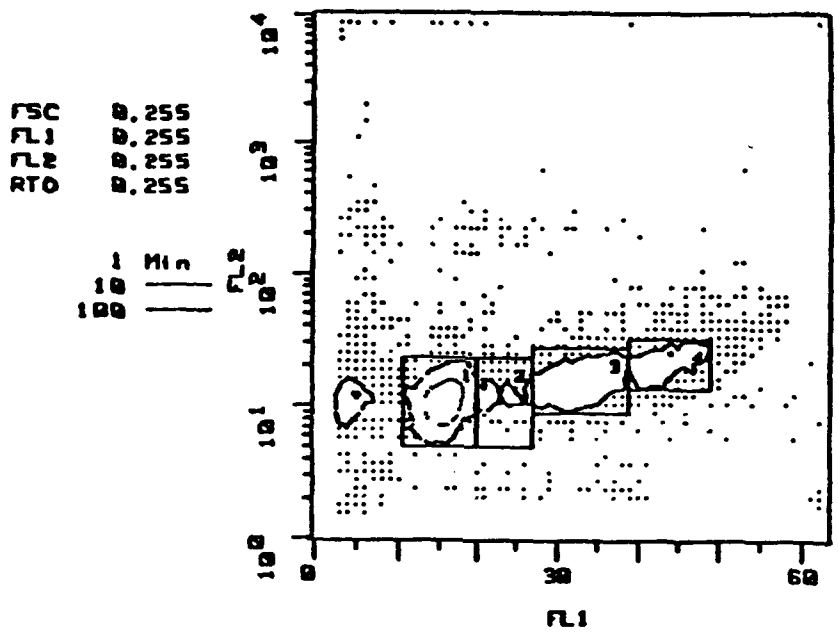


Figure 6. Dual parameter histogram of 244B cells stained with propidium iodide and bromodeoxyuridine. Block 2 relates to S-phase cells and represents 5.9% of cells labelled.

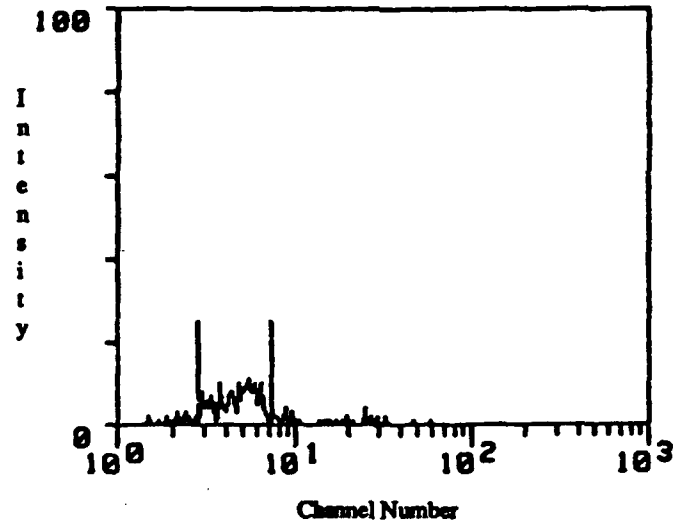


Figure 7. Single parameter histogram of carboxyfluorescein dye in PBS buffer demonstrating low channel signal with very low peak intensity.

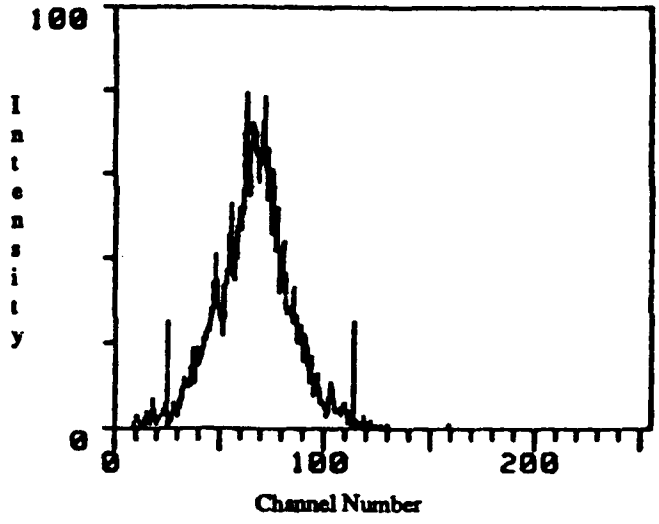


Figure 8. Single parameter histogram of unlabeled 244B cells.

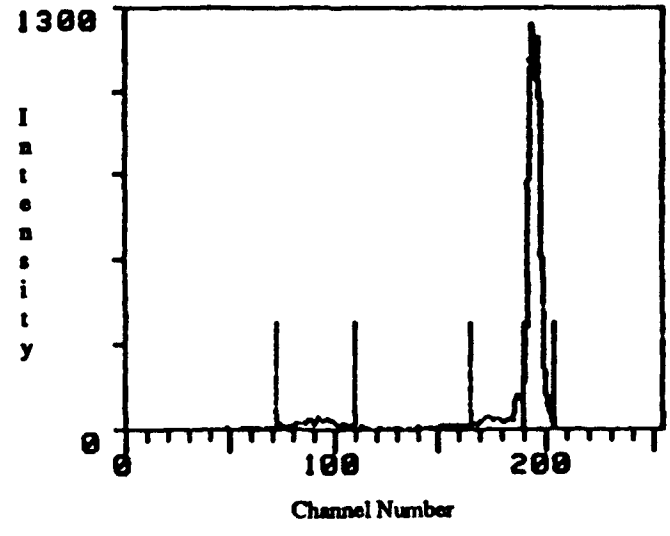


Figure 9. Single parameter histogram demonstrating a change in the main peak channel and peak intensity at 15-min incubation in carboxyfluorescein.

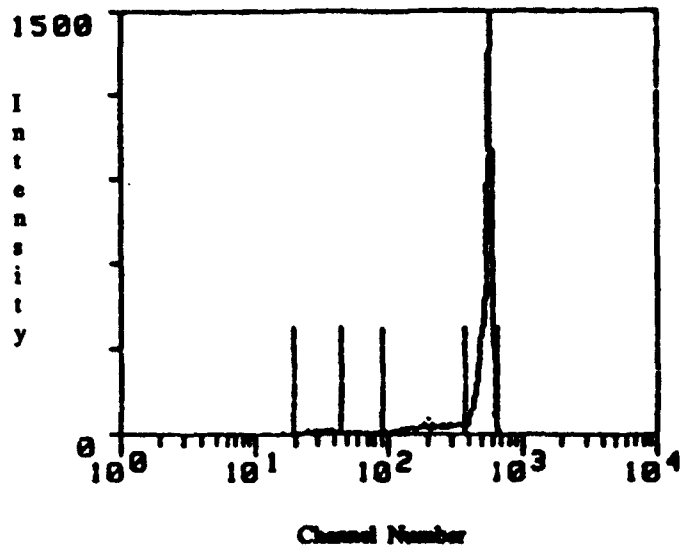


Figure 10. Single parameter histogram showing decreased unlabeled cells and continued increase in peak channel intensity at 20-min incubation.

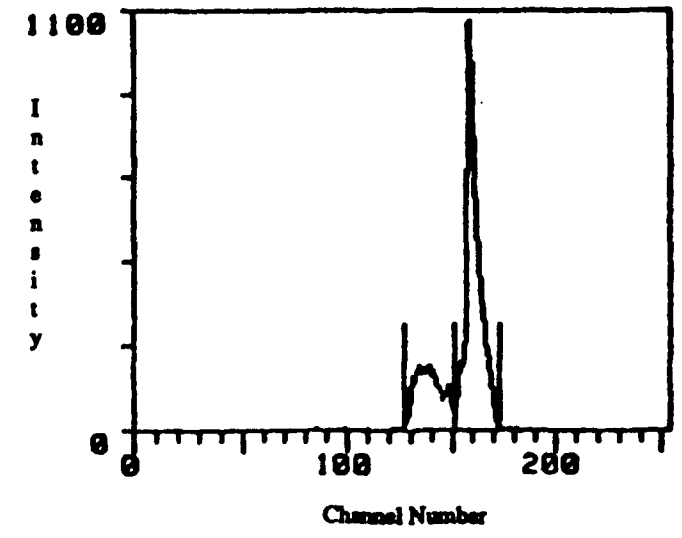


Figure 11. Single parameter histogram indicating a decrease in peak channel intensity after 35 min of incubation with carboxyfluorescein.

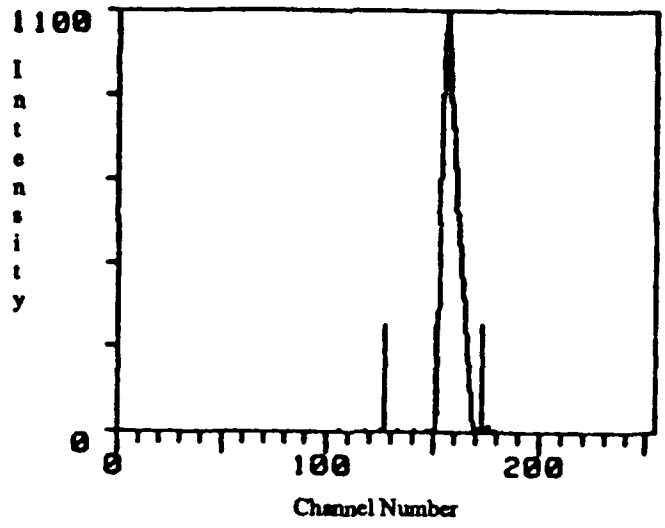


Figure 12. Single parameter histogram of the 50-min carboxyfluorescein incubation.

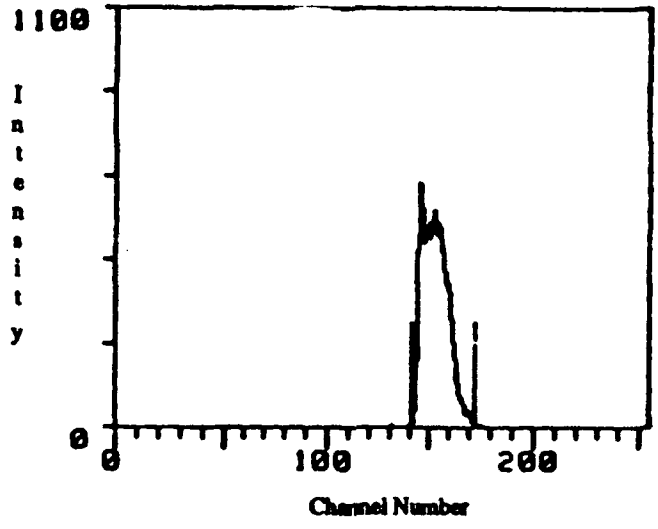


Figure 13. Single parameter histogram of 70-min incubation in carboxyfluorescein. Note the decrease in peak width and intensity.

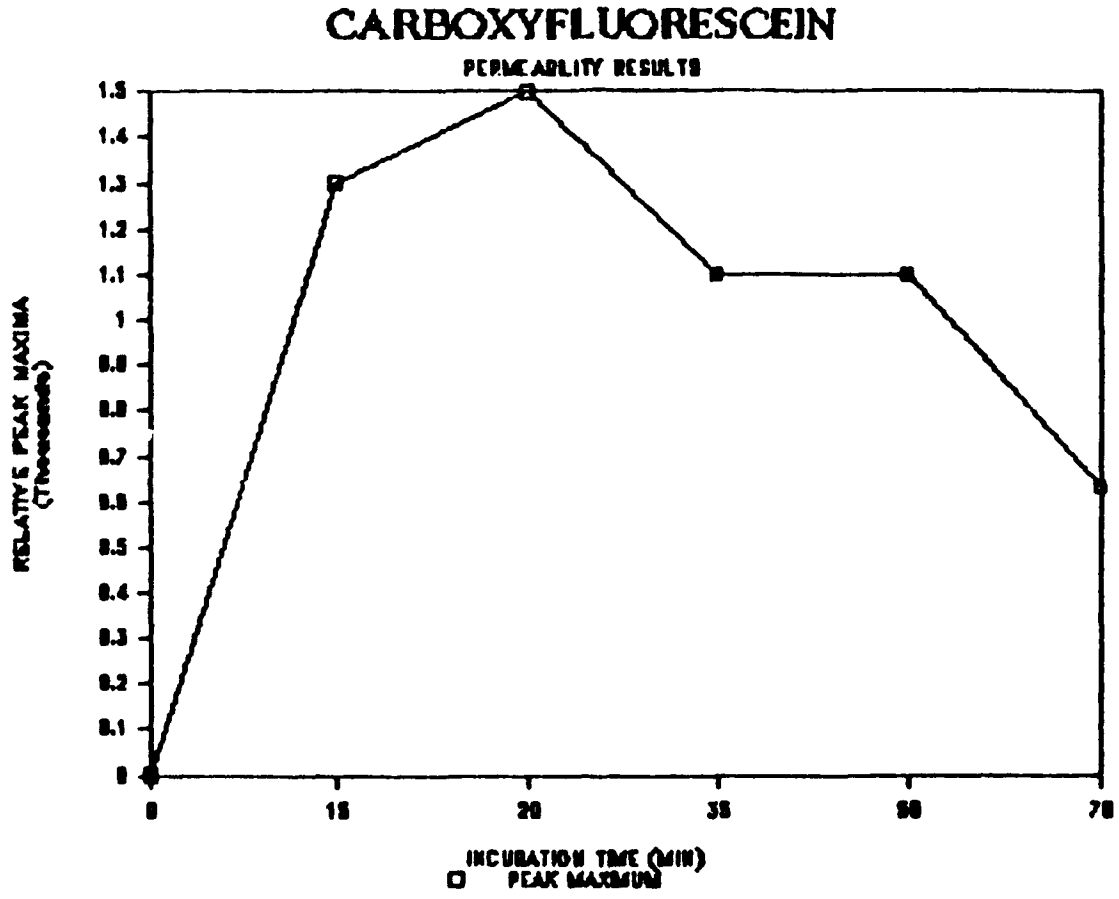


Figure 14. Graphic representation of flow cytometric results from membrane permeability experiments, using dye retention as an indicator of permeability. Note changes in peak maxima vs. incubation time. Following 70-min incubation only 43% of the dye remains in the cell.

TABLE 2. SURFACE MARKER EXPRESSION ON 244B CELLS

Tube Name	Cell Type	% L
Leu 3/2	"Suppressor/Cytotoxic" Cells	2
	"Helper/Inducer: Cells	0
	Ratio 0.0	
Leu 4/HLA-DR	Total T Cells	7
	Activated T Cells	7
	All HLA-DR + Cells	99
	Only HLA-DR + Cells	92
Leu 1/12	B Cells	95
	T Cells	0
	Leu + B Cells	0
Leu 5/11	T Cell (E Rosette Receptor)	0
	Natural Killer Cells	4
Leu 16/B2	B2+ B Cells	44
	Leu 16+ B Cells	83
Calla	Calla+	0
Poly Ig	Poly Ig+	99
IgM	IgM+	14
IgA	IgA+	39
*IgD	IgD+	26
IgG	IgG	89
Kappa	Kappa+	96
Lambda	Lambda+	33
Leu 14/M5	B Cells	12
	Monocytes	1
	Hairy Cells	1
IL-2R	IL-2R+	9
M1/M3	Monocytes	1
	Granulocytes	59
MY7/MY9	MY 7+	5
	MY 9+	0
	MY 9+ MY 7+	2

X-ray Dosimetry

The data from these experiments are presented in Table 3. Using the configuration, operation conditions, and filtration described in the **Materials and Methods** section, we were able to obtain consistent dose rates on the order of 11.28 rads/hour averaged over the 4 flask positions within the air incubator. The data indicate that the field is relatively homogeneous, with some drop-off in dose rate occurring to the left side of the chamber. However, this drop-off is probably due more to the positioning of the air incubator over the X-ray unit than with field inhomogeneity.

TABLE 3. VERY LOW DOSE RATE DOSIMETRY

Sample Position	Dose Rate rad/min ± SE
A	0.2305 ± 0.24
B	0.206 ± 0.07
C	0.22 ± 0.205
D	0.25 ± 0.237
E	0.19 ± 0.106

Air Flow Incubator

The original hole pattern in the air feed tube proved inadequate to provide uniform temperature distribution. These tests were performed using water-filled flasks with temperature probes in place. To remedy this pattern, a minor modification was made to improve air distribution, thereby increasing the homogeneity of the temperatures recorded at the individual flask positions. A flat dispersing baffle was fixed at flask height over the air ports to maximize flow distribution. This modification resulted in a change in the maximum temperature variation between different flask positions inside the incubator from a maximum of 2 ^{o}C to only 0.35 ^{o}C.

Radiation Survival Response of AS52 Cells at Different Dose Rates

The data for a series of 3 independent experiments is summarized in Table 4 and Figure 15. The conventional dose rate used in this study was 1 Gy/min, and the low dose rate was 0.25 Gy/min. The means and standard errors are plotted in the figure.

Response of AS52 Cells After Treatment with the Mutagens Adriamycin and Mitomycin C for 2 or 4 h at Different Concentrations

The results of this investigation are summarized in Figure 16. For the Adriamycin (upper panel), the 2- and 4-h exposures give similar results until a concentration of 0.06 μg/ml is used; the toxicity at this concentration and above is rapid. The data for mitomycin C (lower panel) indicate that the mitomycin C toxicity over the range tested does not significantly change until a concentration of 0.8 μg/ml is used.

Table 4. CHO SURVIVAL DATA: MEANS ± SE FROM 3 EXPERIMENTS

Dose (Gy)	Experiment 1	Experiment 2	Experiment 3
1.06 Gy/min			
2	$4.97\times10^{-1} \pm 2.58\times10^{-2}$	$4.97\times10^{-1} \pm 3.23\times10^{-2}$	$7.66\times10^{-1} \pm 4.84\times10^{-2}$
4	$2.03\times10^{-1} \pm 3.83\times10^{-2}$	$2.24\times10^{-1} \pm 1.31\times10^{-2}$	$3.71\times10^{-1} \pm 3.05\times10^{-2}$
6	$1.12\times10^{-1} \pm 5.34\times10^{-3}$	$4.07\times10^{-1} \pm 1.95\times10^{-2}$	$1.50\times10^{-1} \pm 1.14\times10^{-2}$
8	$3.47\times10^{-2} \pm 3.20\times10^{-3}$	$2.77\times10^{-2} \pm 1.54\times10^{-3}$	$2.31\times10^{-2} \pm 1.37\times10^{-3}$
10	$1.12\times10^{-2} \pm 3.27\times10^{-4}$	$6.24\times10^{-3} \pm 6.46\times10^{-4}$	$7.13\times10^{-3} \pm 2.74\times10^{-4}$
12	$2.53\times10^{-3} \pm 1.45\times10^{-4}$	$3.44\times10^{-5} \pm 1.82\times10^{-5}$	$1.16\times10^{-3} \pm 1.36\times10^{-4}$
0.25 Gy/min			
2	$4.25\times10^{-1} \pm 3.61\times10^{-2}$	$6.35\times10^{-1} \pm 3.45\times10^{-2}$	$4.94\times10^{-1} \pm 2.85\times10^{-2}$
4	$2.55\times10^{-1} \pm 5.73\times10^{-3}$	$2.59\times10^{-1} \pm 2.26\times10^{-2}$	$1.97\times10^{-1} \pm 1.91\times10^{-2}$
6	$1.16\times10^{-1} \pm 6.71\times10^{-3}$		$7.48\times10^{-2} \pm 6.14\times10^{-3}$
8	$3.53\times10^{-2} \pm 3.55\times10^{-3}$	$4.48\times10^{-2} \pm 3.06\times10^{-3}$	$3.28\times10^{-2} \pm 2.51\times10^{-3}$
10	$1.30\times10^{-2} \pm 8.75\times10^{-4}$	$9.65\times10^{-3} \pm 6.27\times10^{-4}$	$1.33\times10^{-2} \pm 1.75\times10^{-4}$
12	$4.23\times10^{-3} \pm 2.29\times10^{-4}$	$2.75\times10^{-3} \pm 2.05\times10^{-4}$	$5.03\times10^{-3} \pm 2.19\times10^{-4}$

The mean values in this table include outliers which were not included in the averages used in pooled data in the summary figure.

Survival of AS52 Cells After 2- or 4-h Incubations with Adriamycin or Mitomycin C at 37 °C and 40 °C

The results of these experiments are shown in Figure 17. For the Adriamycin, a 2-h treatment at .075 μg/ml showed increased toxicity at 40 °C (vs. 37 °C). For a 4-h treatment, a similar increase in toxicity was observed for the .055 μg/ml concentration at 40 °C. At .01 μg/ml, no toxicity was observed at either temperature.

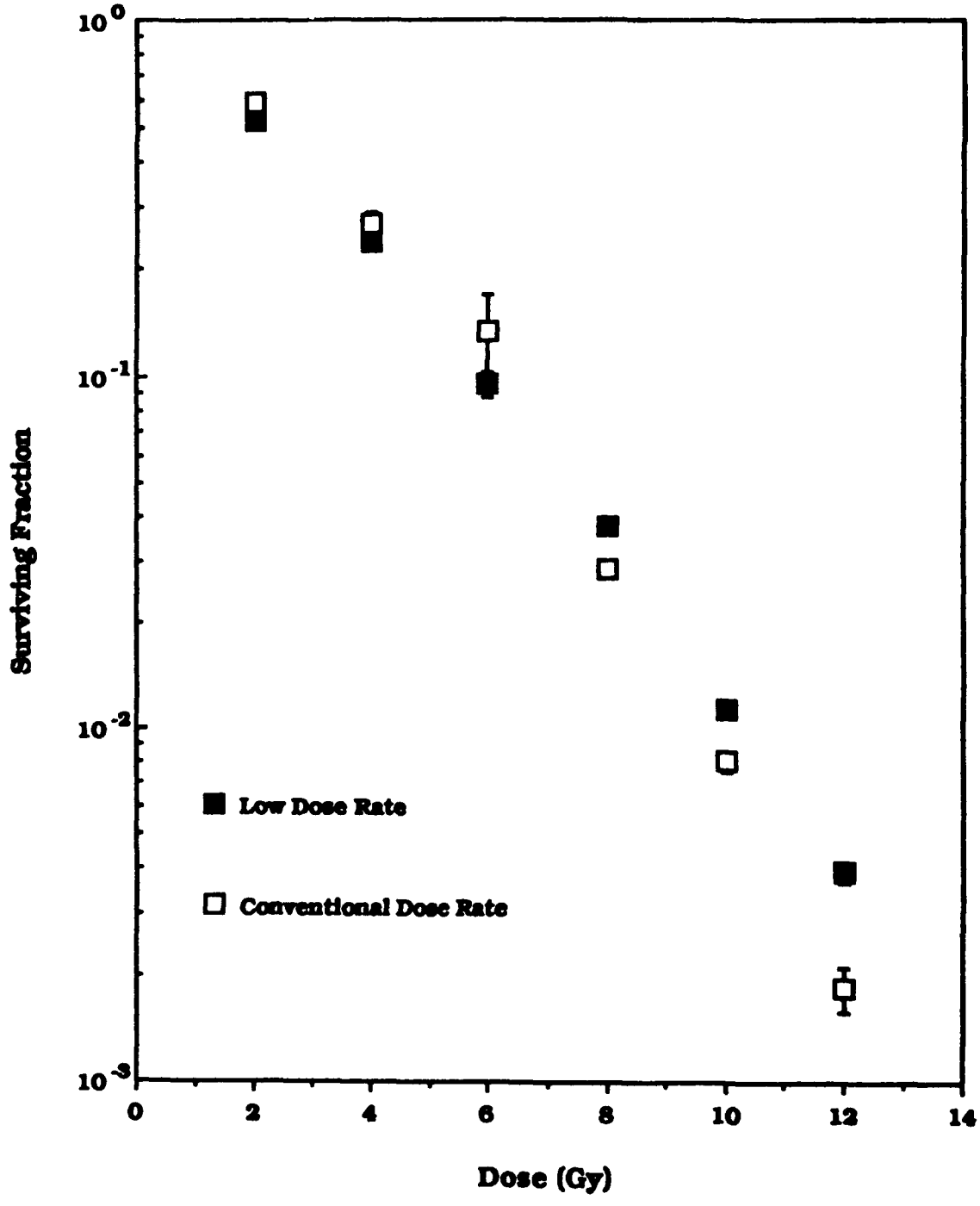
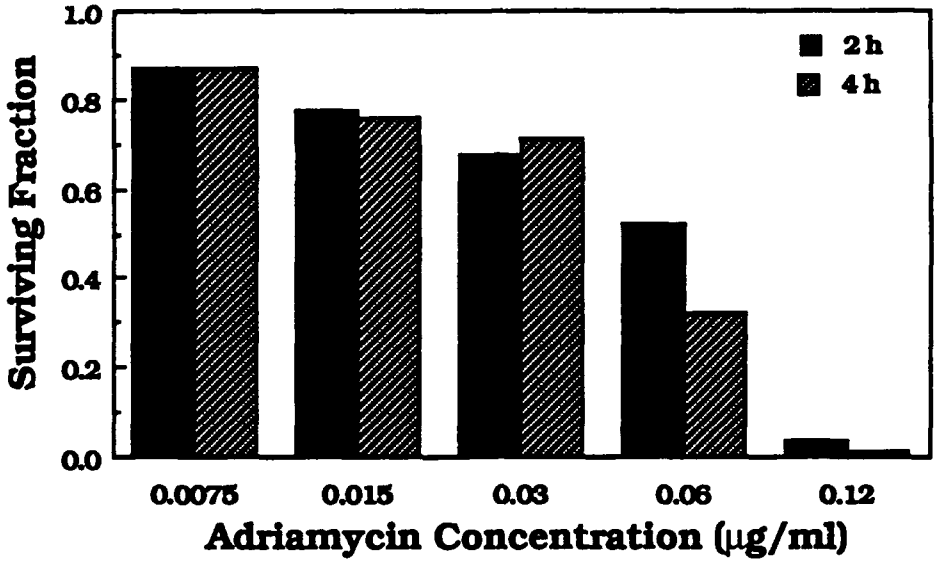


Figure 15. Survival of AS52 Chinese hamster cells after exposure to orthovoltage x-rays at conventional (1 Gy/min) and low (0.25 Gy/min) dose rates.



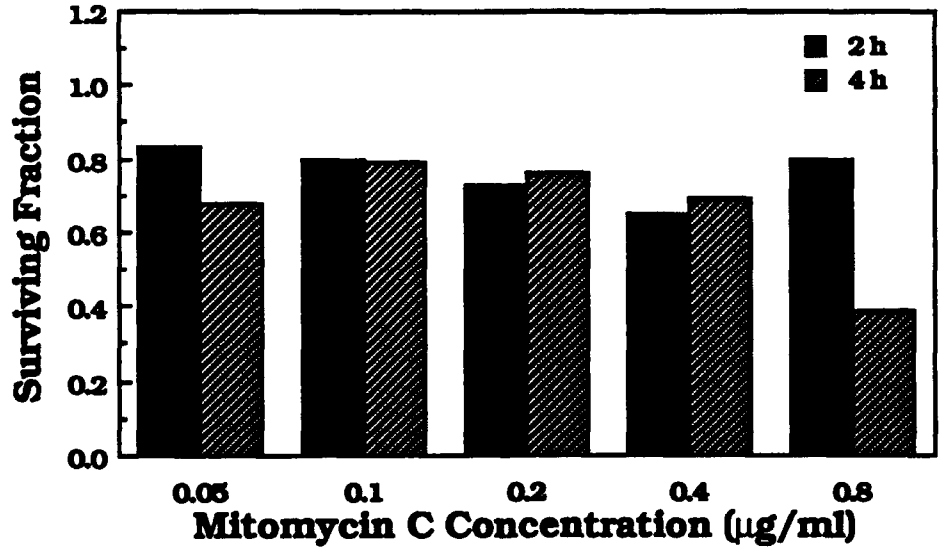


Figure 16. Survival of AS52 Chinese hamster ovary cells after exposure to Adriamycin (upper panel) or mitomycin C (lower panel) for 2 or 4 h at 37 °C.

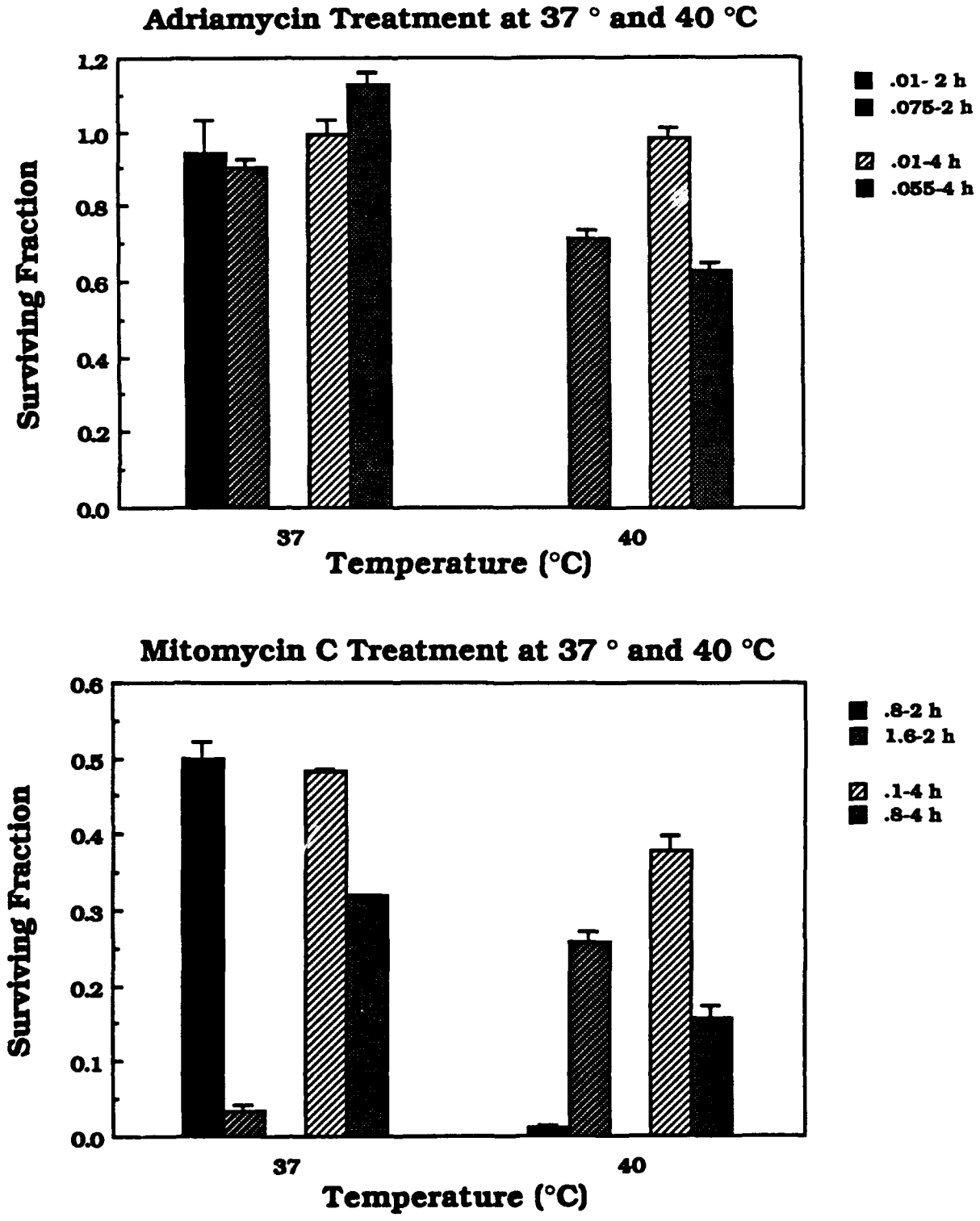


Figure 17. Survival of AS52 Chinese hamster cells after treatment with Adriamycin (upper panel) and mitomycin C (lower panel) at 37 °C and 40 °C.

For the mitomycin C (MMC), treatment at 0.8 μg/ml at 37 °C is toxic, with an apparently very large increase in toxicity at 40 °C. The data at 2 h, for a concentration of 1.6 μg/ml of MMC, are questionable. For the MMC and 4-h exposure, at both concentrations tested (0.1 and 0.8 μg/ml), an increase in toxicity was observed at 40 °C.

Comparison of the Survival of the AS52 Cell Line and Its Parent BH4 Cell Line After Hyperthermic Treatment at 42 °C and 42.5 °C

The results of a single experiment comparing the survival response of the AS52 cell line, and its parent BH4 cell line, are shown in Figure 18. Apparently from this data, the AS52 cell line is more sensitive (at each incubation time) than its parent line at both of these temperatures. This data was not confirmed in a second experiment, and, therefore, remains to be studied further.

DISCUSSION

Surface Markers on 244B Cells

B-cell development follows a well-defined path with regard to immunoglobulin expression. The pre-B cell initially has only cytoplasmic mu heavy chain proteins and does not express either kappa or lambda light chains. Further, it does not express any surface immunoglobulins. The pre-B cell will progress toward maturity with the expression of surface IgM and later of both IgM and IgD. Along with these immunoglobulins, other surface markers appear during the developmental sequence. CD 10, 19 and HLA-DR appear in the early pre-B stage. CD 10 is lost and CD 11 and CD 20 appear later, while the cells are still in the pre-B stage. CD 21 and CD 16 (the IgG Fc receptor) are detected on circulating B cells along with CD 11, 19, 20 and HLA-DR. With activation to a functional Ig secreting cell comes a concomitant increase in the expression of CD 23 and CD 25. As the cell differentiates toward the plasma cell stage, there is a loss of pan-B markers. The presence, on 59% of the 244B cells tested, of the granulocytic cell marker CD 15 did at first cause some confusion. To ascertain whether or not we had a contaminated cell line or a unique marker appearing, we entered into discussions with the cells developer (Dr. J. Schwartz) and with Dr. J. Kiel (U.S. Air Force School of Aerospace Medicine (USAFSAM , now Armstrong Laboratory). Possibly, this expression could be a unique marker on the 244B cells, as opposed to contamination. This expression is based on discussions with Dr. Kiel as to the plausibility that the culture conditions stressed the cell to the point of activation, with the possible expression of this particular marker. Based on studies that Dr. Kiel has conducted, it is possible that our culture conditions may not adequately protect the cells from oxygen radical formation, and this may in turn be causing the (possible) differentiation of our cell line. Pending further discussion with Dr. Schwartz at the University of Chicago, no further marker studies have been attempted.

Membrane Permeability

The results appear to indicate that by 70 min of incubation, the carboxyfluorescein dye is beginning to leach from the cells. If we use peak maxima as an indicator of the relative amount of carboxyfluorescein within the cells, the 20-min dye-loading incubation would be the optimum time point for

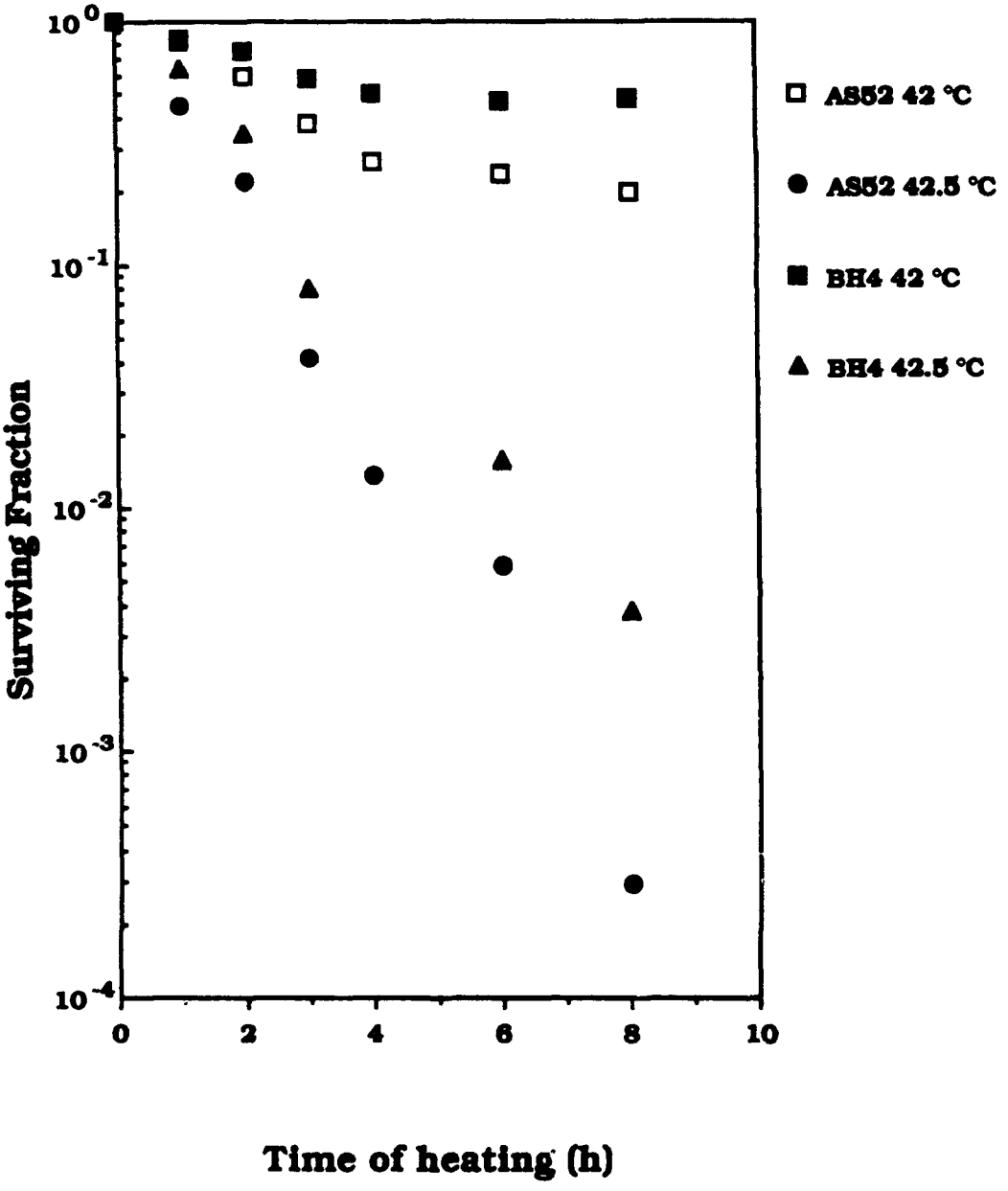


Figure 18. Comparative survival of AS52 and parent BH4 Chinese hamster ovary cell lines heat-treated at 42 °C and 42.5 °C.

cell loading. The decrease in label seen at the 70-min time point would indicate that more than half of the label which had initially entered the cells had subsequently leaked out of the cells. If this is true, then incubations of preloaded cells for up to 4 h, which would be met during the proposed microwave/X-ray exposure experiments on cell membranes, could be expected to yield less than optimal results.

Radiofrequency Radiation Induction of Chromosome Aberrations in CHO Cells

In this type of study, the 37 °C control will give a baseline value for the "spontaneous" occurrence of chromosome aberrations in CHO cells. The temperature control (TC) serves to indicate whether any effect observed for RFR is due to the RFR, or simply due to elevating the cells to a higher temperature (using a water bath).

As can be seen in the information summarized in Table 5, for pulsed wave exposures at 800 MHz and 1,200 MHz, no evidence exists that RFR induces chromosome aberrations. There were no statistically significant differences between the number of chromosome aberrations in the RFR exposed cells and the 37 °C or TC cells.

Radiation Survival Response of AS52 Cells at Different Dose Rates

As expected, the data in Figure 15 indicate that the AS52 cells show increased survival when the dose rate of the Orthovoltage X-rays is lowered below 1 Gy/min. This increase is especially obvious at the higher doses (above 8 Gy).

Response of AS52 Cells After Treatment with the Mutagens Adriamycin and Mitomycin C for 2 or 4 h at Different Concentrations

The results presented in Figure 16 indicate that for studies examining the potential interaction between microwaves or hyperthermia and the chemical mutagen Adriamycin, the treatment concentrations selected (of the chemical) would be below 0.1 μg/ml. For such exposures involving mitomycin C, the treatment concentration selected would be in the range of 0.4 to 0.8 μg/ml.

Survival of AS52 Cells After 2- or 4-h Incubations with Adriamycin or Mitomycin C at 37 °C and 40 °C

These experiments provided us with preliminary information for selecting treatment concentrations of the chemical mutagens Adriamycin or mitomycin C, for use in mutation experiments involving simultaneous microwave exposures and/or hyperthermia treatments at 40 °C. Our goal is to treat at 40 °C with a level of chemical which will result in about 10% to 60% survival. These experiments indicate that for a 2-h exposure to Adriamycin, we will need concentrations of .075 μg/ml or above, while for a 4-h exposure, we would need an Adriamycin concentration of .055 or above. For the mitomycin C, for a 2-h exposure, the 0.8 μg/ml concentration would be acceptable, with a high of 1.6 μg/ml still being useful, while for a 4-h exposure, we would want a concentration slightly lower than 0.1 μg/ml, and the 0.8 μg/ml concentration would be useful.

Comparison of the Survival of the AS52 Cell Line and Its Parent BH4 Cell Line After Hyperthermic Treatment at 42 °C and 42.5 °C

The data from this experiment remain to be confirmed in additional experiments. This study has been undertaken because in earlier studies, the AS52 cell line appeared to lack the thermotolerance seen in the wild type CHO cell line. We, therefore, are comparing the response of the AS52 cell line to its immediate parent cell line.

Table 5. ABSENCE OF INDUCTION OF CHROMOSOME ABERRATIONS BY 850 MHz AND 1,200 MHz PW RFR

850 MHz PW

Experiment 1	ABERRATION TYPE (PER 100 CELLS)						
Experimental Condition	BRK	RAD	ID	DIC	RF	AC	N
37 °C Control	4	0	0	0	0	28	500
RFR Exposed	0	0	0	2	0	16	300
Temperature Control	0	0	0	4	0	34	400
Adriamycin Treated	2	0	0	0	0	0	214

Experiment 2	ABERRATION TYPE (PER 100 CELLS)						
Experimental Condition	BRK	RAD	ID	DIC	RF	AC	N
37 °C Control	0	0	0	0	0	18	400
RFR Exposed	8	0	0	0	0	10	400
Temperature Control	4	0	0	2	0	8	400
Adriamycin Treated	14	0	0	0	0	32	400

Table 5 (Continued)

1.2 GHz

Aberration Type (Per 100 Cells)

Experimental Condition	BRK	RAD	ID	DIC	RF	AC	N
37 °C Control	4	0	0	0	0	8	500
RFR Exposed	0	0	0	0	0	0	400
Temperature Control	0	0	0	0	0	4	400
Adriamycin Treated	2	0	0	0	0	6	300

Summary of abbreviations: BRK = chromosome break; RAD = radicals; ID = deletion; DIC = dicentric; RF = ring plus fragment; AC = acentric fragments and double minutes; and N = number of cells scored

REFERENCES

Ciaravino, V., M.L. Meltz, and D.N. Erwin. Effects of Radiofrequency Radiation and Simultaneous Exposure with Mitomycin C on the Frequency of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells. Environ Mutagenesis 9:393-399 (1987).

Kerbacher, J.J., M.L. Meltz, and D.N. Erwin. Influence of Radiofrequency Radiation on Chromosome Aberrations in CHO Cells and Its Interaction with DNA Damaging Agents. Rad Res 123:311-319 (1990).

Krishan, A. Rapid Flow Cytofluorometric Analysis of Mammalian Cell Cycle by Propidium Iodide Staining. J Cell Biol 66:188-193 (1975).

Lee, PS, and C.J. Rudd. A New Procedure for Quantitation of Mutations at the HGPRT Locus in Chinese Hamster Ovary (CHO) Cells. Submitted for Publication, Envir and Molec Mutagenesis (1991).

Meltz, M.L., P. Eagan, and D.N. Erwin. Absence of a Mutagenic Interaction Between Microwaves and Mitomycin C in Mammalian Cells. Environmental and Molecular Mutagenesis 13:294-303 (1989).

Meltz, M.L., P. Eagan, C.R. Harris, and D.N. Erwin. Dosimetry Considerations in Far Field Microwave Exposure of Mammalian Cells. Physiol Chem and Phys and Med NMR 20:23-30 (1988).

Meltz M.L., K.A. Walker, and D.N. Erwin. Radiofrequency (Microwave) Radiation Exposure of Mammalian Cells During UV-Induced DNA Repair Synthesis. Rad Res 110:255-266 (1987).